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DYSREGULATED CIRCADIAN RHYTHM PATHWAY IN HUMAN OSTEOARTHRITIS: NR1D1 AND BMAL1 SUPPRESSION ALTERS TGF-B AND IL-1B SIGNALING IN CHONDROCYTES

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Purpose: Circadian rhythm (CR) was identified by RNA sequencing as the most dysregulated pathway in human osteoarthritis (OA) knee articular cartilage. This study examined circadian rhythmicity in cultured chondrocytes and the role of the CR genes NR1D1 and BMAL1 in regulating chondrocyte functions. NR1D1 is a negative regulator of BMAL1, a core clock gene that regulates circadian rhythmicity.
Methods: RNA was extracted from human cartilage tissues harvested from normal and OA knees (n=14 each), and expression levels of NR1D1 and BMAL1 mRNA were assessed by quantitative PCR. NR1D1 protein expression was confirmed by immunohistochemistry in normal and OA human cartilage, as well as in normal knees and knees with surgically induced OA of mice. To examine the circadian rhythmicity of gene expression in cultured chondrocytes isolated from normal human cartilage, chondrocytes were synchronized by dexamethasone and harvested at 4-hour intervals up to 48 hours for RNA and protein extraction. Chondrocytes were then treated with small interfering RNA (siRNA) for NR1D1 or BMAL1, followed by IL-1β or TGFβ stimulation to test the effect of knock down on response to IL-1β and TGFβ.
Results: Both NR1D1 and BMAL1 mRNA levels were significantly reduced in OA cartilage, compared to normal cartilage. NR1D1 protein was predominantly expressed in the superficial and upper-mid zone of normal cartilage, both in human and in mouse knees. The protein expression was reduced in OA cartilage, although high expression was observed in cluster cells. In surgically induced OA in mice, NR1D1 protein expression was significantly reduced before cartilage damage occurred. In cultured human chondrocytes, a clear circadian rhythmicity was observed for NR1D1 and BMAL1 mRNA levels. NR1D1 expression was at its lowest level at T12 and T36, whereas highest expression was observed at T24 and T48. The expression pattern of BMAL1 displayed the reversed pattern. Treatment with siRNA significantly suppressed levels of both genes at all time points, but the rhythmic expression pattern was preserved for NR1D1. Increased BMAL1 expression was observed at T24 and T48 after knocking down NR1D1, and decreased NR1D1 levels were observed at all time points after knocking down BMAL1. IL-1β treatment significantly induced IL6, COX2, iNOS, MMP13 and ADAMTS4. NR1D1 knock down further increased the expression levels of iNOS, MMP13 and ADAMTS4, while by contrast the IL-1 induction of IL6 and COX2 was blunted. Genome-wide sequencing of RNA from chondrocytes treated with NR1D1 and BMAL1 siRNA identified 330 and 68 genes, respectively, that were significantly different. TGFβ signaling pathway was affected by both siRNAs. NR1D1 and BMAL1 knock down increased the expression of elastin and tenascin C, following induction by TGFβ.
Conclusions: The circadian rhythm pathway is dysregulated in OA cartilage. Interference with circadian rhythmicity in cultured chondrocytes affects IL-1β and TGF-β signaling, which are central pathways in cartilage homeostasis and OA pathogenesis.

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DIO2-KNOCKOUT MODULATES CIRCADIAN CLOCK GENES IN ARTICULAR CARTILAGE THROUGH THYROID HORMONE SIGNALING

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Purpose: Previously, the type II deiodinase (D2) gene (DIO2) has been identified as relevant OA susceptibility gene. D2 regulates the bioavailability of intracellular triiodothyronine (T3) and expression of DIO2 mRNA and D2 protein levels in osteoarthritic cartilage are increased as compared to healthy cartilage. Given the function of T3 signalling this should be considered detrimetnal to cartilage integrity. In this respect, we showed that knocking-out Dio2 expression in C57Bl/6 mice, has a protective effect particularly after administrating a stringent running regime. Here, we aim to identify intrinsic underlying differences in articular cartilage tissue homeostasis between wild-type- and Dio2-knockout-mice.
Methods: Genome wide gene expression data (Illumina MouseWG-6 v2) of knee-cartilage of wild-type and C57Bl/6-Dio2 -/- -mice was used to detect the involved gene expression pathways. To increase power in our analysis, probes with a |fold-change| ≥ 1.5 between wild-type- and knockout-mice were analyzed and subsequent multiple test correction according the "bonferroni-method" was performed. STRING-db and STITCH analysis was applied to visualize protein-protein (PPI) and protein-chemical interaction (PCI)-networks.
Results: Comparing the microarray-data of articular cartilage of knockout- and wild-type-mice showed 69 probes that were differentially expressed with a |fold-change| ≥ 1.5 whereas 4 probes, representing 4 genes remained significant after subsequent multiple testing correction. Notably, two of these genes are known canonical genes, involved in the circadian clock rhythm. Except for Calr (FC = -1.73; Padj. = 0.00050), direct protein interaction (PPI) was found between LOC100047427 (Predicted: similar to thyroid hormone receptor (Thra); FC = 1.74; Padj. = 0.0331) and circadian clock genes, Dbp (FC = 2.61; Padj. = 0.0264) and Nr1d1 (FC = 1.57; Padj. = 0.0307) (Figure 1A). To visualize the interrelationship of this PPI with respectively Dio2, T3 and its receptor Thra, we next added these in a STITCH-db analysis (Figure 1B) and confirmed interaction of this PPI with thyroid signaling. Furthermore, ChAP-seq experiments performed by Chatonnet et al; (2013) showed that the promoters of these genes are under influence of the thyroid hormone receptor (THR)-alpha (Dbp) and -beta (Nr1d1) in mice.
Conclusions: We demonstrated that Dio2¬-depletion in articular cartilage results in significant higher expression of circadian clock genes Nr1d1 and Dbp, likely mediated via the thyroid hormone receptors. Furthermore, our data demonstrates that upregulation of circadian rhythm genes could, in part, explain the previously established beneficial effect of articular cartilage tissue homeostasis of Dio2 knockout mice.

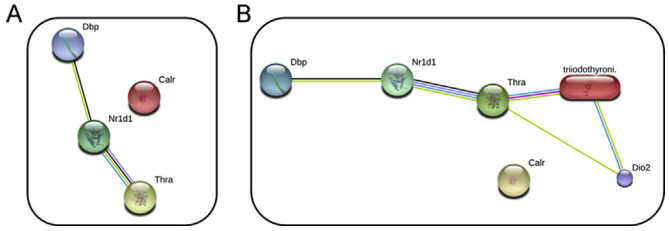

Figure 1. Gene networks in search tool for the retrieval of interacting genes. (A) Screenshot of the PPI-network for the 4 genes significantly differing >|1.5|-fold between knockout- and wild-type-mice (STRING-db). (B) Screenshot of the PCI-network for the 4 genes significantly differing >|1.5|-fold between knockout- and wild-type-mice, including Dio2 and triiodothyronine (T3) (STITCH).

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INCIDENT BISPHOSPHONATE USE AND RISK OF KNEE REPLACEMENT SURGERY AMONG WOMEN WITH INCIDENT KNEE OSTEOARTHRITIS

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Purpose: Bone remodeling as a therapeutic target in knee osteoarthritis (OA) has gained much interest, but the effects of antiresorptive agents on knee OA have been conflicting. Since mechanical factors play a central role in knee OA and bone adapts readily to mechanical stresses, it is important to understand the effects of modulating bone remodeling via antiresorptive agents such as bisphosphonates on the course of knee OA. We assessed the relation of incident bisphosphonate use to the risk of knee replacement (KR) surgery among women with incident knee OA.
Methods: We used data from The Health Improvement Network (THIN), a general practitioner (GP) electronic medical records database representative of the UK general population. We included women aged 50-89 with incident knee OA between 1/1/2003-12/31/2012 who had ≥1 GP visit or ≥1 prescription during the one year prior to the knee OA diagnosis. We excluded women with existing KR (prior to incident knee OA or bisphosphonate use), those less likely to be KR candidates (BMI>40, history of joint infection, high risk cancers (pancreatic, esophageal, gastric or other metastatic), dialysis, supplemental oxygen,